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DECREASED SERUM TRYPTOPHAN IN PATIENTS WITH CANCER CACHEXIA CORRELATES WITH INCREASED SERUM NEOPTERIN

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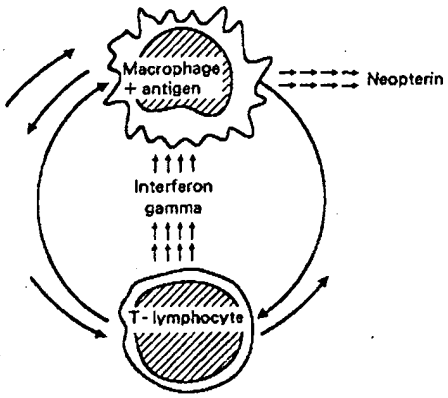
ABSTRACT

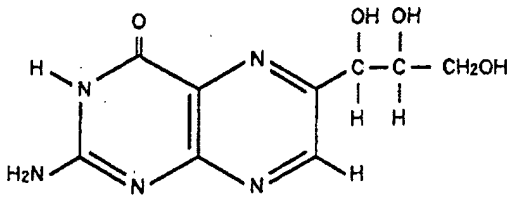
We investigated serum tryptophan (Trp), neopterin (NPT) and immunosuppressive acidic protein (IAP), one of tumor-associated tumor marker, concentrations in 28 patients with gastrointestinal tumors representing cancer cachexia and 10 healthy controls. NPT comes from activated macrophages presumably activated by tumor-sensitized T cells via gamma-interferon (IFN-γ) excitation of the macrophages. We found that the NPT level was significantly higher than the control value. The negative correlation of NPT and Trp concentrations indicates activity of indoleamine 2,3-dioxygenase(IDO), a Trp degradating enzyme, in cancer-burden patients. The activity of IDO can be induced by cytokines such as IFN-γ, and therefore low Trp levels may result from endogenous IFN-γ production due to immune activation against tumors. We also found a positive correlation between NPT and IAP levels, suggesting that host immune activation against tumors played a role in the immunosuppression of cancer-burden states, followed by cancer-cachexia.

INTRODUCTION

Tryptophan (Trp) is an essential and indispensible aminoacid required for biosynthesis of proteins, serotonin (a neurotransmitter), the pineal hormone melatonin and a major source of the vitamin niacin (1). Intake of less than the

required amount promptly results in a negative nitrogen balance and significant reductions in Trp metabolite levels in the blood (2). Because of the Trp released from the breakdown of body protein during Trp deprivation, it is probably not possible to decrease serum Trp below a certain level, as long as body protein pools are available for breakdown. Metabolism of Trp to the niacin pathway is initiated in the liver by Trp dioxygenase. The excellent work of Osamu Hayaishi's group showed the presence of another distinct nonhepatic enzyme able to form kynurenin from Trp, which was named indoleamine 2,3-dioxygenase (IDO) (3,4,5,6,7). Most exciting were reports that IDO was highly induced by stimulation of the immune system with bacterial endotoxins (3,8), by virus infection (9) or by gamma-interferon (IFN-γ) (7,10,11). Independently, it was shown that the antitumor effect of IFN-γ results from depletion of Trp by induced IDO *in vitro* and *in vivo* (12,13). In recent years, neopterin (NPT: 6-D-erythro-[1',2',3' -trihydroxy-propyl]-pterin) was identified as a new biochemical marker for the activation of cellular immunity. In mixed cultures of human peripheral blood mononuclear cells, production of NPT was described for the first time in an *in vitro* system (14). IFN-γ stimulates the key enzyme, guanosine-triphosphate(GTP) - cyclohydrolase I, of NPT biosynthesis in human macrophages (15), and human macrophages activated by IFN-γ were then identified as the cellular source of raised NPT production (16). IFN-γ is produced by activated T cells and, in turn, activates macrophages. So NPT elevation directly points to stimulation of macrophages and indirectly to T-cell activation, and therefore demonstrates activation of the cellular immune system or increased endogenous IFN-γ production (Fig.1, Fig.2). In this research, we have investigated whether low Trp levels and high NPT concentrations could be demonstrated in patients with gastrointestinal tumors. We also performed the simultaneous determinations of carcinoembryonic antigen (CEA), a tumor-specific tumor marker, and immunosuppressive acidic protein (IAP), a tumor-associated tumor marker. IAP was first found to be a type of alpha 1-acid glycoprotein.in the ascitic fluids of





[chemical structure of neopterin]

Fig 1. The interaction of T-cell and macrophages resulting in neopterin release

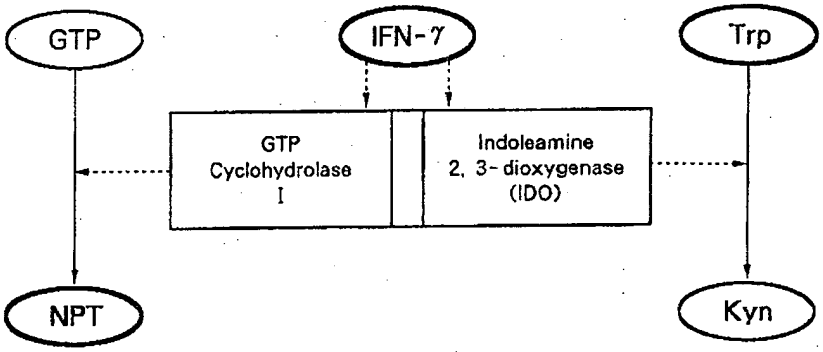


Fig. 2. The relationship between IFN-γ, NPT and Trp metabolism. Abbreviation : IFN-γ= gamma-interferon, NPT = neopterin, Trp = tryptophan, Kyn = kynurenin, GTP = guanosine triphosphate IDO = indoleamine 2, 3-dioxygenase

cancer patients. Shibata reported that macrophages produced IAP when they were stimulated by either immune complex or inflammatory agents such as endotoxin *in vitro*. Its biochemical properties are significantly different from those of acidic protein in the serum pf normal persons. Previous studies have indicated that the serum IAP concentration increases in most cancer patients and decreases to a normal level as such patients are cured. Thus, the monitoring of

serum IAP aids in planning and assessing clinical staging and is a good follow-up tool in cancer patients (17,18). Relationship between CEA and IAP and NPT were also analyzed and discussed herein.

PATIENTS AND METHODS

Serum neopterin (NPT), tryptophan (Trp), carcinoembryonic antigen (CEA) and immunosuppressive acidic protein (IAP) were synchronously measured in a total of 56 specimens of 28 patients with unresectable and noncurative-operative malignant tumors and 10 healthy controls. Clinical features of the patients with tumors are shown in Table 1. Prognostic nutritional index (PNI) was calculated as the following formula ; PNI = 10 x serum albumin (g/dl) + 0.01 x peripheral lymphocyte count (per mm^3). PNI was reported to be effective as a prognostic indicator and is useful in clinical practice as a determinant of the multimodal treatment of cancer patients. PNI of the poor prognostic group was significantly lower than that of the good prognostic group and the control group (19). In this research, cancer-burden patients were divided into two groups; PNI >40 and PNI <40. The group of PNI <40 indicates severe malnutrtional states and that of PNI >40 indicates moderate malnutritional states. NPT concentrations in serum were measured by high pressure liquid chromatography (20,21) and Trp levels were measured by aminoacids autoanalyzer (HITACHI 835-50, JAPAN). Single radial immunodiffusion was used for the quantitative assay of serum IAP levels (22) and radioimmunoassay for CEA levels. Both IAP and CEA levels were measured with a commercially available immunoassay. Data were reported as mean (SD) and statistically analyzed by Student's *t* test, analysis of variance and determination of coefficient of correlation, as appropriate.

RESULTS

[1] Dependence of NPT and Trp concentrations on PNI

Serum NPT concentrations were significantly ($p < 0.01$) higher in patients with malignant tumors compared with normal subjects. Moreover, patients of

Table 1. Clinical characteristics of patients with cancer cachexia

No. of patients (No. of specimens)		28 (56)
Prognostic Nutritional Index (PNI)	PNI : > 40	8 (16)
	PNI : < 40	20 (40)
Male : female		14 : 14
Median age in years (range)		58 (28 - 88)
Tumor types		
Colorectal		13
Gastric		9
Hepatocellular		4
Cholangiocellular		1
Pancreatic		1
Dissemination		
Liver		11
Peritoneum		9
Paraaortic lymphnode		3
Ovary		3
Bone		2
Lung		2
Skin		1
Therapy		
Chemotherapy		28
Immunotherapy		4

PNI is a prognostic indicator and calculated as the following formula ; PNI = 10 x serum albumin (g/dl) + 0.01 x peripheral lymphocyte count (/mm^3).
PNI 40< and PNI <40 indicate severe and moderate malnutritional state, respectively.

PNI <40 had significantly higher serum NPT concentrations compared with those of PNI >40 . These results indicate that serum NPT concentrations correlated with severity of malnutrition due to cancer cachexia. In contrast, serum Trp levels of the patients were significantly ($p<0.01$) lower than the healthy control value. Patients of PNI <40 had significantly lower serum Trp levels than those of PNI >40 (Fig. 3). These results indicate a negative association of NPT concentrations and Trp levels.

[2] Correlation between NPT concentrations, CEA and IAP levels

Serum NPT concentrations and CEA levels were not correlated ($r=0.11$, $p<0.05$), but serum NPt concentrations and IAP levels were positively correlated ($r= 0.73$, $p<0.05$) (Fig. 4).

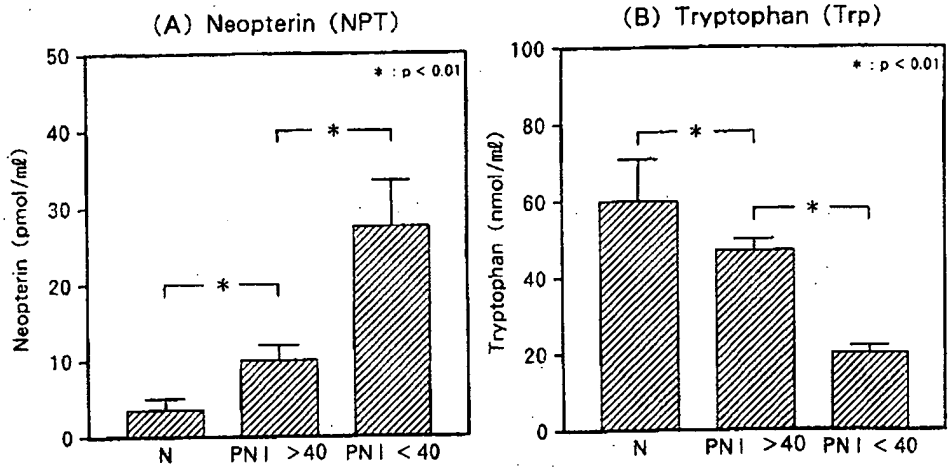


Fig 3. Dependence of NPT and Trp concentrations on PNI
Abbreviation : NPT = neopterin, Trp = tryptophan, N = normal subjects
PNI = prognostic nutritional index

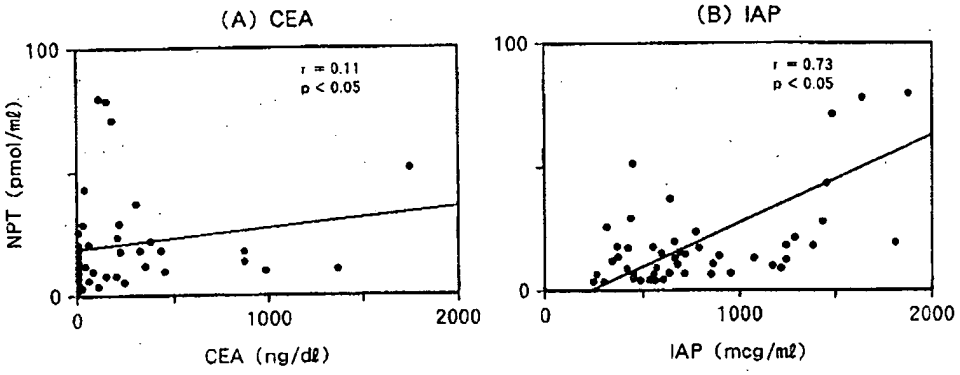


Fig 4. Correlation between NPT concentrations, CEA and IAP levels.
Abbreviation : NPT = neopterin, CEA = carcinoembryonic antigen,
IAP = immunosuppressive acidic protein,
r = coefficient value of correlation, p = probability

DISCUSSION

We demonstrated that large amounts of NPT were produced by human macrophages on stimulation with IFN-γ in cancer-burden patients (14,15,16). *In vitro* data showed that IFN-γ is also able to induce indoleamine 2,3-dioxygenase (IDO) which degrades Trp to kynurenine (7,10,11). In our patients with cancer cachexia, increased activity of IDO is indicated by decreased serum Trp levels which confirms earlier *in vitro* results (3,4,5,6,7).

A negative association existed between NPT and Trp, which supports the concept that immune activation and increased degradation of Trp may be the reason for reduced Trp levels, rather than reduced dietary intake. The dependence on the nutritional status indicated further induction of IDO and further catabolism in the progression of cachexia. These results suggest that serum NPT concentrations reflect clinical status of cancer cachexia or disease severity of cancer-burden patients.

Impairment of cellular immune responses to tumor elimination is common in hosts bearing progressively growing malignant tumors (20,21). Studies of patients with progressively growing malignant tumors have shown depressed cutaneous hypersensitivity to many recall antigens (22).This immunodepression has been attributed to the presence of a variety of soluble factors such as tumor antigens, tumor-specific antigen-antibody immune complexes, and immunoregulatory proteins in the circulation of tumor - bearing hosts (23,24,25,26). One of these serum factors has been purified and characterized according to its physicochemical properties and immunosuppressive activities, and this serum factor was designated immunosuppressive acidic protein (IAP) (27,28,29).

The relationship between NPT and IAP is illustrated in Fig. 4, in which NPT concentrations are plotted against IAP levels for individual specimens. The estimated correlation (r value) was 0.73, which is highly significant. As described above, the measurement of serum NPT can be considered an indirect evaluation of cell - mediated immunity. The evidence of a significant association

between increase of NPT concentrations and elevation of IAP levels would suggest that host immune - activation play a role in the immunosuppression of the cancer - burden patients. This hypothesis also supports the fact that cytokines produced by activated - lymphocytes not ony stimulate an effective antitumor - cytolytic response but also regulate the degree of proliferative capacity of tumor cells (30).

No significant correlation was found between NPT concentrations and CEA levels (Fig.4). As described above, the cellular source of NPT is IFN-γ – stimulated macrophages. Therefore, our results clarify that increased NPT levels in cancer - burden patients are a reflection of the activation of the host's immune system against cancer.

The immunosurveillance theory refers to the activation of cell - mediated immunity which produces a beneficial effect, but shows diminished *in vitro* responses to mitogen by the cells from the cancer patients. This seemingly paradoxical behaviour is explained by the fact that increased NPT concentrations in cancer - burden patients indicate an immunological activation which does not necessarily imply immune - mediated destruction of tumor cells.

An explanation to this paradoxical relationship of immune activation and depressed response to mitogen in cancer - burden patients can be proposed by the following model: extreme cell - mediated immune activation results in immune depression and/or immune dysfunction. In this model, NPT concentrations may have a potential possibility to be a parameter implicating the subtle balance of the immune response. In our present findings, there is a hint that may unlock a hidden aspect of the immunosurveillance theory.

Elevation of NPT concentrations was detected in a variety of diseases in which immune activation is known to be an actor, such as rejecting allografts, infecting viruses and microbes, causing autoimmune diseases and infection with human immunodeficiency virus type I (HIV - I) (31,32,33,34). In all these diseases, T - cell responses in vitro are typically diminished, possibly by the *in vivo* presence of cytokines due to persistent immune activation (35,36).

The defective *in vitro* response of T - cells to soluble antigens was shown to be associated with raised NPT concentrations *in vivo* in early stages of HIV - I infection (34). Recent researches revealed that elevated NPT concentrations are associated with a subsequent decrease in CD4+ T - cell counts and serving as a strong predictor of the progression of AIDS (37,38). Thus, accumulated data on patients with HIV - I infection demonstrate that immune activation with elevated NPT concentrations may coexist with functional defects of immune effector mechanisms.

In conclusion, production of endogenous IFN - γ is increased in the course of tumor progression due to activation of cell - mediated immunity against tumor proliferation. Endogenous IFN - γ induced indoleamine 2,3 - dioxygenase (IDO) activity, a Trp degradating enzyme, which results in a loss of Trp levels. The catabolism of Trp by IDO can be reasonably expected to influence protein synthesis as well as production of serotonin and niacin (1).

Additionally, a number of other secondary or indirect effects may result. Niacin is a putative angiogenic factor (39). Trp or one of its pyridine metabolites was shown to be necessary for IFN - γ – mediated induction of tumor cell cytotoxicity in macrophages (40). Serotonin may influence the release of prolactin from the pituitary (41), and a recent report indicates that prolactin is important for macrophage activation and T - cell function (42) . Perhaps related to these effects is the report that serotonin may also influence TNF-mediated anti-tumor activity (43).

At an immunological level, serotonin reduces IFN-γ induced expression of HLA-Ia surface antigens on mouse macrophages (44), competes with muramyl peptides for receptor binding (45), and influences immune responses (46). Serotonin also acts as a growth factor for intestinal mucosal cells (47) and blockage of serotonin synthesis by inhibition of biopterin synthesis leads to intestinal necrosis in mice which is relieved by administration of tetrahydrobiopterin, the cofactor for hydroxylation of Trp (48).

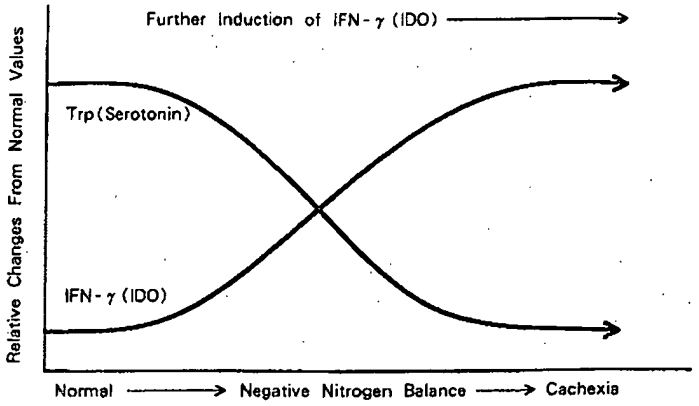


Fig 5. Predictive changes in Trp(serotonin) and IFN-γ (IDO) resulting from immune response in cancer-burden state.
Abbreviation : Trp = tryptophan, IFN-γ = gamma interferon, IDO = indoleamine 2, 3-dioxygenase

Fig. 5 suggests the hypothesis of cancer cachexia. If this hypothesis is correct, then it also suggests therapeutic or at least palliative approaches to the management of cancer cachexia, i.e. restoration of Trp levels either by Trp supplementation, or by prevention of Trp catabolism by IDO. However, in view of reports of markedly enhanced toxicity of Trp in rodents pretreated with LPS (49,50) and the risk of elevating kynurenin levels (Fig. 2), which induces mental and metabolic disorders, simple trials of Trp supplementation in humans who may have elevated IDO levels will have to proceed with caution.

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